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Molecular and Epigenetic Impacts of Pesticides on the Female Reproductive System: A Review

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Abstract: *Pesticides, primarily originating from agricultural activities, have raised environmental and health concerns due to their suspected effects on the female reproductive system. Despite their widespread application, a significant research void exists concerning their implications for human female reproductive health. This review provides comprehensive information on the adverse effects of pesticides on female reproduction, focusing on their molecular and epigenetic mechanisms. In general, these adverse outcomes include ovarian dysfunction, hormonal irregularities, the onset of endometriosis, and an increased risk of stillbirths. From a mechanistic perspective, pesticides can interfere with the endocrine system, catalyze the formation of reactive oxygen species, and induce oxidative stress. At the molecular level, certain pesticides can act as hormone mimics, disrupting the normal hormonal balance. Moreover, they can trigger epigenetic shifts, leading to alterations in non-coding RNAs, histone configurations, and DNA methylation patterns. These epigenetic alterations not only have an immediate impact but can also be passed on to subsequent generations. Highlighting these concerns, this review underscores the urgent need for stringent regulations and aims to catalyze future in-depth studies on the relationship between pesticides and female reproductive health.*

Keywords: *Fungicide; Insecticide; Herbicide; Organochlorine; Reactive oxygen species; Epigenetic*

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1. INTRODUCTION

The female reproductive system plays an important role in mammalian species, in such functions as sexual activity, fertility, pregnancy, childbirth, and breastfeeding. Its anatomical structures comprise the uterus, ovaries, fallopian tubes, cervix, and vagina. The health and well-being of this system are determined by a variety of internal and external factors, ranging from lifestyle choices to environmental exposures [1]. Consumption of several diets can improve the quality of the female reproductive system, especially the ovaries [2]. However, other factors reduce the quality of the female reproductive system. Notably, certain occupational exposures, especially radiation, heat, and potent chemicals, escalate the risks of numerous diseases, including, premenstrual syndrome, infertility, endometriosis, and polycystic ovary syndrome [3-5]. Moreover, contaminants like plasticizers, heavy metals (e.g., lead, mercury, and arsenic), and pesticides, further intensify these risks [6].

Pesticides, crucial for maintaining agricultural productivity, are formulated to

mitigate threats from pests during cultivation, storage, and transportation. These chemical agents can be categorized based on their target, ranging from insecticides and rodenticides to more niche types such as molluscicides and nematicides [7-9]. Despite their extensive use in both residential and farming environments, they have significantly adverse effects on the environment and various organisms. These chemical residues not only contaminate water sources but also enter the food chain, posing direct and indirect risks to organisms. Organisms can encounter these toxins through skin contact, inhalation, and food consumption [10, 11]. As these chemicals persist in the environment, their potential for bioaccumulation intensifies, transforming organisms into toxic reservoirs and affecting the greater food chain [12].

Specifically concerning the female reproductive system, the consequences of pesticide exposure are multifaceted, ranging from immediate repercussions to transgenerational epigenetic impacts. When exposure transpires during pregnancy, the placenta, a protective barrier for the fetus, often fails to guard the developing child from pesticide exposure, potentially leading to developmental disruptions[13]. Additionally, during lactation, neonates can indirectly absorb these toxins through breast milk, potentially hindering their development [14]. Some of these consequences could persist, with the potential of being passed down to future generations, manifesting as epigenetic modifications [15, 16]. Considering the broad spectrum of pesticides currently in use and the limited in-depth research on their effects on female reproductive health, this review aims to elucidate the specific mechanisms of molecular pathways and epigenetic alterations these pollutants induce in the female reproductive system.

2. MATERIALS AND METHODS

This review used literature to gather information regarding the effect of pesticides pollution resulting from agricultural activities on the female reproductive system. The primary portal for general searches was Google Scholar (https://scholar.google.com/). This platform facilitated the identification of key concepts associated with the female reproductive system. For a more refined literature search, bibliographic databases (i.e., Scopus and PubMed) were also utilized to identify related issues. Searches within these bibliographic databases were conducted using the keywords: "Agricultural", "Pesticide", "Reproduction", and "Female". To streamline the identification and evaluation of relevant articles, certain inclusion criteria were established: articles had to be written in English, and they had to fall within the study timeframe of 1975-2023. The exclusion criteria applied during this process encompassed articles not in English and those that didn't directly address the effects of pesticide exposure on the female reproductive system.

RESULTS AND DISCUSSION

Category of Agricultural Pesticides

In the past five decades, pesticides have played a pivotal role in ensuring food security [17]. These chemicals, used in agricultural fields, private gardens, and public spaces, are designed to control pests. Pesticides can be grouped by their target organism and their chemical makeup. Notably, classifications from the Globally Harmonized System (GHS) and the World Health Organization (WHO) also categorize pesticides based on their toxicity or potential adverse effects [18]. Based on their targets, pesticides can be segmented into insecticides, fungicides, herbicides, and rodenticides [19]. Chemically, they are divided into four primary types: organochlorine, organophosphate, carbamate, and pyrethroid [20].

Organochlorine compounds contain five or more chlorine atoms and were the first synthesized insecticides used in agriculture. Based on their long-lasting environmental impact, examples of these insecticides include DDT, lindane, endosulfan, aldrin, dieldrin, heptachlor, toxaphene, and chlordane [21]. Derived from phosphoric acid, organophosphates include common pesticides like parathion, malathion, dichlorvos, diazinon, and glyphosate. Carbamates, stemming from organic carbamic acid, encompass compounds like carbaryl, carbofuran, and aminocarb [22]. These function similarly to organophosphates, targeting nerve signal transmission in pests, leading to their eventual poisoning and death [23].

The physiological effects of pesticides on the female reproductive system

Effect on the physiological quality of the ovary

Ovarian function is closely related to egg formation, ovulation, and the menstrual cycle. The process of folliculogenesis occurs in the ovary. The process of folliculogenesis begins with the development of a number of small primordial follicles, secondary, tertiary (antral), and pre-ovulatory (graafian) follicles, into large preovulatory follicles [24]. Finally, the ovulation process occurs by excreting the oocyte. The corpus luteum that is left behind can produce the hormone progesterone. This hormone has complex functions, including developing breast tissue, preparing the uterus for pregnancy, and protecting against endometrial cancer in postmenopausal women. The work of pesticide compounds can interfere with the performance of the ovaries (Table 1). The disrupted egg cell formation process can result in infertility in women [25]. Toxic compounds from pesticides are one of the disruptive agents for the female reproductive system. Heptachlor is an organochlorine compound that acts as an insecticide. The effect of heptachlor on ovarian cells is that it can reduce estradiol concentrations [26]. In addition, heptachlor can interfere with and prolong the estrus cycle. The mechanism of action of heptachlor is interference with hormone synthesis, so it can cause ovarian cycle irregularities [27].

Linuron and dimethomorph are a group of herbicides. Herbicides, such as Linuron and dimethomorph, can cause ovarian dysfunction and lead to infertility in women. Linuron and dimethomorph work by Inhibiting androgen signaling during crucial ovary phases of development. The folliculogenesis process is disrupted so that the follicle maturation stage will be hampered. Boberg et al., 2023 showed that the two mechanisms of action that are affected are androgen receptor (AR) and aromatase (CYP19A1) inhibition [28]. Direct impacts on the AR in the ovaries may cause key signaling pathways that control follicle development to become disrupted, reducing the number of available competent follicles. Indirectly, ovarian dysfunction can be caused by decreased LH/FSH release. This decrease occurs due to disturbances in the neuronal circuit organization in the brain. This indirect pathway is the mechanism of action of aromatase inhibition.

The malathion compound is a type of insecticide with the organophosphate group. Malathion has been studied to be toxic to the female reproductive system. Malathion is a type of organophosphate pesticides that can reduce female fertility. Malathion has the effect of reducing healthy follicles by increasing the incidence of atresia in the ovaries. Malathion works by inducing apoptosis in granulosa cells from antral follicles in sheep ovaries (caprine). Granulosa cell death will cause condensation of chromatin, nuclear loss, damage to mitochondria structure, and increased vacuolization by lipid droplets. The mechanism of cell biochemical action that occurs involves the formation of oxidative stress. This oxidative stress can increase lipid peroxidation [29].

Fenoxaprop-ethyl (FE) is a group of herbicides that are still under-researched because of its toxic properties. There is a toxic effect of FE on oocyte quality. However, this pesticide is already widely used in agriculture. The results of research on rats exposed to FE showed a decrease in ovarian weight and oocyte count. In addition, oocytes with low quality are indicated by a reduction in the rate of first-polar body extrusion and fertilization ability. The results also show that the oocyte has Actin expression and abnormal meiotic spindle morphology. Overall, the mechanism of action of FE on cells is apoptosis, autophagy, and mitochondrial dysfunction because of reactive oxygen species (ROS) accumulation [30].

Table 1: *Effect of various types of pesticides on female reproduction*

Pesticide types	Pollutant pesticides	Impact mechanisms	Organisms	Impacts on the female reproductive system	References
Fungicide	Organochlorine pesticide	Endocrine disrupting	Monkey	• Reduces menstrual cycles • Interfere with estradiol concentrations at ovulation	[31]
	Hexachlorobenzene (HCB)	Endocrine disrupting	Rat	Indirect effect on steroidogenesis	[32]
Insecticide	Heptachlor-Organochlorine pesticide	Endocrine disrupting	Rat	• Suppresses progesterone and oestradiol concentrations in blood • Decrease oestradiol production by the ovarian cells	[33]
Insecticide	Dichloro- diphenyl-trichloroethane (DDT)	Endocrine disrupting	Rabbit	Decrease progesterone concentrations during early pregnancy	[34]
			Human	Abnormal ovarian structure	[35]
			Monkey	Decreased oestradiol, progesterone, and chorionic gonadotrophin concentrations	[36]
Insecticide	DDT+Lindane	ROS generation and oxidative stress	Rabbit	Reduced ovulation rate	[34]
	3,3',4,4'-tetrachloroazoxybenzene (PCB)	Endocrine disrupting	Rat	Increased oestrous cycle length	[37]

Pesticide types	Pollutant pesticides	Impact mechanisms	Organisms	Impacts on the female reproductive system	References
Herbicide	Tetrachlorodibenzo-p-dioxin (TCDD)	Endocrine disrupting	Rat	• Hormone cycle irregularities-longer duration of dioestrous • Inhibits ovulation	
			Mice	Endometriosis	[38]
Insecticide	Methoxychlor-Organochlorine	ROS generation and oxidative stress ER-signaling pathway	Mice	• Reduce ovulated eggs • Ovulation defects in prenatal and neonatal • Induced follicle atresia • Ovulation defects	[39,40]
		Inhibition of the development of gap junctions between cumulus and oocyte cells	Rat	• Follicular development inhibition • Reduce antral follicle • Increase in pre-antral follicles	[41,42]
			Rat	Induces preimplantation embryonic loss	[43]
Multiple ability (Insecticide, Herbicide, Fungicide, Algacide)	Pentachlorophenol	Endocrine dysfunction at hypothalamic	Human	Ovarian or adrenal insufficiency-Infertility	[44]
Herbicide	Dioxin	Endocrine disrupters	Monkey	Endometriosis- Uterine lining	[45]

Pesticide types	Pollutant pesticides	Impact mechanisms	Organisms	Impacts on the female reproductive system	References
Insecticide	DDE 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene	Immunoreactive vascular endothelial growth factor (VEGF)	Rat	Polycystic ovaries and impaired fertility	[46]
Herbicide	Atrazine	Endocrine disrupting action	Pig	Extended diestrus with regular cycling, increased ovarian follicular cysts, and decreased ovarian weight	[47,48]
Insecticide	DDT	Endocrine disrupting action	Rat	• Prolonged estrus • Absence of corpora lutea • Ovarian hypertrophy	[49]
Fungicide	Mancozeb/Maneb	ROS generation and oxidative stress	Rat	• Reduced healthy follicles • Decreased of corpora lutean the rat ovary	[39,50]
Insecticide	Malathion Organophosphate	Oxidative stress-initiating granulosa cell apoptosis within healthy follicles	Caprine	• Increases follicular atresia • Ovarian follicle loss • Alteration in the estrous cycle	[51]
Insecticide	Chlorpyrifos	Endocrine disrupter	Rat	• Reproductive dysfunction • Suppressing reproduction hormones • Reduced number of implantation	[52]
Insecticide	Endosulfan	Oxidative stress	Mice	• Reduced in ovarian follicles and corpora lutea • Increase in atretic follicles	[53]

Pesticide types	Pollutant pesticides	Impact mechanisms	Organisms	Impacts on the female reproductive system	References
Insecticide	Endosulfan +Malathion	Free radicals-MDA level	Rat	• Decreases the size and number of healthy follicles • Increase the number of atretic follicles	[54]
Insecticide	Methidathion	Rise in ROS level	Rat	Reduced healthy follicles	[55]
Insecticide	Chlorpyrifos	Endocrine disrupter	Human	Reduced FSH level in the follicular phase	[56]
Herbicide+ Fungicide	Linuron and Dimethomorph	Genes downregulated to cell proliferation and androgen receptor antagonism and/or aromatase inhibition	Rat	• Impaired follicle growth • Follicle maturation delay	[28]
Insecticide	Imidacloprid	Oxidative stress	Rat	• Decrease estrous cycle • Increased diestrous • Decreased healthy follicles • Increased atretic follicles	[57,58]
Herbicide	Fenoxaprop-ethyl (FE)	ROS generation-mitochondrial dysfunction, apoptosis, and autophagy	Mice	• Reduced number of oocytes and ovary weight • Decrease rate of first polar body extrusion	[30]
Insecticide	Lambda-Cyhalothrin (LCT)	Embryonic resorption	Rat	• Stillbirth • Reduce postnatal survival	[59]
Insecticide	Diethylphosphate	Pyrethroids toxicity	Human	Shortened gestations age delivery	[60]
Insecticide	Beta-cypermethrin (β-CYP)	Reduction in the PI3K/Akt/mTOR pathway	Rat	Downregulation of endometrial stromal cell proliferation and differentiation	[61]

Effect on the endometrium

One of the abnormalities in the endometrium is endometriosis. Endometriosis occurs due to the endometrial tissue exists outside the uterus. Endometrium can grow on the inner lining of the stomach (peritoneum), ovaries (ovaries), intestines, vagina, or urinary tract. This condition causes irritation or inflammation of the tissue around the endometrium. This disease is correlated with estrogen. This is because estrogens have been known to stimulate the growth of endometrial lesions. This disease leads to infertility. Studies on exposure to chronic dioxin treatment showed significant results in increasing the incidence of the development of endometriosis [62].

Effect on the fetal implantation and birth

Women working in the agricultural sector with regular exposure to pesticides are at risk of spontaneous abortion and stillbirth [63,64]. One of the pyrethroid pesticides used to repel pests in agriculture is Lambda-Cyhalothrin (LCT). Administered Lambda-Cyhalothrin (LCT) to rats showed significant results in decreasing total litter birth weight and increasing stillbirth. Iheanacho et al. 2020 states that the study disagreed with the WHO report due to the results of the study (1989). The WHO report (1989) stated that the treatment of low doses of cypermethrin had no negative effects on body weight and some reproductive parameters in female rats, mice, and rabbits. In contrast, the results of the study by Iheanacho et al. 2020 including several studies showing that cypermethrin treatment with low doses has adverse effects in fetuses including induced pre and post-implantation losses, embryonic resorption, weight gain in fetuses, and number of viable fetuses [65-67]. The difference in the results of the research indicates that further research on the effects of pesticides on reproductive organs, especially in humans, is urgently needed. This is due to the widespread use of pesticides and the very high risk of accidental exposure. Moreover, the effect of pesticide exposure on time to pregnancy has a negative correlation. Women who work in flower greenhouses are at risk of exposure to high levels of pesticides. This causes a long time to achieve pregnancy [68,69]. However, it is necessary to carry out research on the types of pesticides that have an effect and how the mechanism of action forms in pregnancy and increasing stillbirth.

Molecular mechanism of the pesticides on female reproductive system

Next, we focus on the molecular mechanism of the pesticides toxicity on the reproductive system. In general, the pesticides affected the female reproductive system by acting as Endocrine Disrupting Chemicals (EDCs). Pesticides can act as EDCs by mimicking the chemical structure of the hormones, blocking the hormonal actions, as well as altering the hormone levels in the bloodstream (Table 2). In human and rats, organochlorines like endosulfan [70], and DDT is reported to mimic the estrogens and can bind to several receptors, i.e., estrogen receptors (ER), aryl hydrocarbon orphan dioxin receptor (AHR), glutamate receptor alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA). Binding of DDT to ER, AHR, and AMPA can increase the GnRH activity, which leads to early maturation in rat's female reproductive system [71-73].

DDT is also reported to induce changes in gene expression via ER-independent pathways, through p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinases 1/2 (ERK1/2) [74-76]. In human embryonic kidney (HEK293) and human endometrium (Ishikawa) cells, phosphorylation of p38 by DDT upregulates the AP-1 activity by inducing the formation of c-Jun/c-Fos dimers, which activates the collagenase promoter [76]. In human breast cancer (MCF-7) cells, o,p'-DDT upregulates the vascular endothelial growth factor A

(VEGFA) by inducing p38 kinase phosphorylation, which activates CREB-binding protein (CBP) binding to Hypoxia-inducible factor 1 (HIF-1). The binding of this complex to the VEGFA promoter at the HIF-1 response element (HRE), increase the VEGFA transcription [74].

In Japanese quail, Kamata et al. reported that o,p'-DDT decreased the production and distribution of calcium-binding proteins osteopontin (SPP1), calbindin D28k (CALB1), and transient receptor potential cation channel subfamily V, member 6 (TRPV6) in the uterus, which leads to the thinning of the [80,81]. Meanwhile, in domestic hen, eggshell thinning by o,p'-DDT exposure is mediated by the disruption of the carbonic anhydrase (CA) activity [82].

Herbicide atrazine is reported to inhibit the pulsatile release of GnRH in female rats, probably by affecting the kisspeptin and neurokinin B neuropeptides in the dynorphin neurons [77].

Meanwhile, diaminochlorotriazine decreased the LH release in murine LβT2 cells by interfering the caffeine-sensitive ryanodine receptor which reduced the GnRH-mediated calcium uptake/storage and LH release [78].

While organochlorines can act as EDCs by mimicking the estrogen and interact with ER, fungicide prochloraz act as ER antagonist which downregulates the ERα and ERβ mRNAs expression [79]. Fungicide mancozeb is also reported to downregulates the ERβ and integrin β3 of Ishikawa cells which leads to lower attachment of human primary endometrial epithelial cells (EECs) and human blastocyst surrogate trophoblastic spheroids (JEG-3) [83], that explains the spontaneous abortion and irregular menstruation observed in women as well as inhibition of implantation in female mice exposed to mancozeb [83-87].

Table 2: *Pesticides as EDCs and its affected receptors*

Type of pesticides	Pesticides	Organism	Key Receptors, Enzymes and Affected Hormones	References
Organochlorine	Endosulfan	Rat	ER	[70]
Organochlorine	DDT	Rat	ER, AHR, AMPA, GnRH	[71,72]
Organochlorine	DDT	Japanese quail	SPP1, CALB1, TRPV6	[80,81]
Organochlorine	DDT	Chicken	CA	[82]
Organochlorine	DDT	Human cells	ER	[73]
Organochlorine	DDT	Human cells	p38 MAPK, ERK1/2	[74-76]
Herbicide	Atrazine	Rat	Kisspeptin and neurokinin B neuropeptides (putative), GnRH	[77]
Herbicide	Diamino-chlorotriazine	Mouse cells	Caffeine-sensitive ryanodine receptor, LH	[78]
Fungicide	Prochloraz	Human cells	ERα and ERβ	[79]
Fungicide	Mancozeb	Human cells	Erβ and integrin β3	[83]

Abbreviations: AHR: aryl hydrocarbon orphan dioxin receptor; AMPA: alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; CA: carbonic anhydrase; CALB1: calbindin D28k; ER: estrogen receptor; GnRH: gonadotropin-releasing hormone; MAPK: mitogen-activated protein kinase; SPP1: osteopontin; TRPV6: transient receptor potential cation channel subfamily V, member 6.

Epigenetic mechanisms of the pesticides on female reproductive system

The phenotypic effects of pesticides on female reproduction have been discussed above. In this section, we will focus on the downstream effects (i.e ., epigenetic alterations) of pesticides on the female reproductive system (Table 3). The pesticides discussed are categorized into insecticides, herbicides, and fungicides. Three insecticides have been reported to influence epigenetic alterations in the reproductive organs of female rodents, i.e., methoxychlor (MXC), endosulfan, and chlordecone (CD) [88-90]. Besides insecticides, other pesticides such as herbicides (i.e., atrazine (ATZ), glyphosate, and fenoxaprop-ethyl (FE)) and fungicides (i.e., vinclozolin and mancozeb) are also associated with epigenetic changes in the reproductive systems of various organisms [30, 91-94].

Epigenetic alterations refer to the modification of DNA, RNA, and its associated proteins without DNA sequence changes. These alterations include non-coding RNAs, histone modifications, and DNA methylation. DNA methylation is the addition or removal of methyl groups to DNA molecules, which play a role in the gene expression regulation. Histone modifications refer to the alterations of various chemical groups in histone protein, such as acetyl, methyl, phosphate, or ubiquitin. These alterations affect the expression of genes due to the accessibility of DNA during transcription. Non-coding RNAs can be categorized into microRNA (miRNA), long non-coding RNAs (lncRNAs), and small interfering RNAs (siRNAs), which are not translated into proteins [95,96]. Although not translated, non-coding RNAs are critical in regulating post-transcriptional genes, regulating transposons, and preventing viruses.

Alterations in histone modifications, DNA methylation, and non-coding RNAs represent the changing of downstream gene expression associated with female reproduction, resulting in the developmental and functional disruption of tissues and organs in the female reproductive system. Female reproductive organs, such as the ovary and uterus, have been found to have epigenetic effects due to exposure to pesticides. The epigenetic changes not only affect individuals but are also passed down from one generation to multiple subsequent generations.

Epigenetic effects of pesticides on the ovary

The epigenetic effects on the ovaries due to pesticide exposure have been demonstrated in rodents (i.e., rats and mice), and medaka (Oryzias latipes). Methoxychlor (MXC), an insecticide, was exposed to the F0 generation of pregnant rats from embryonic day (E) 19 to postnatal day (PND) 7, and epigenetic properties were analyzed from adult ovaries. Hypermethylation of DNA is observed in estrogen receptor beta (ERβ) promoter regions, but no alterations in the methylation of estrogen receptor alpha (ERα). ERα and ERβ are nuclear receptors expressed in female reproductive organs, which are responsible for mediating the effect of estrogen hormone. To ensure the DNA methylation changes, the DNA methyltransferase (DNMT) level was also measured. The level of DNMT3B increased with the administrated dose of MXC (100 mg/kg/day). These series of DNA methylation results were followed by the increase of the ERβ [97]. In another report, a higher dose of MXC (200 mg/kg/day) was also given to pregnant rats during the gestational day (GD) 8 – 14, resulting in ovary diseases at F0 generation. In addition, the disease not only occurred individually but throughout generations (F3) [89].

Another insecticide that demonstrates the effect on ovary epigenetics was chlordecone (CD). The administration of 100 µg/kg/day of CD to rats during gestation could alter specific histone modifications, i.e., increased H2Aub and H3K27me3, decreased H4ac and H3K4me3 in embryonic oocytes, and

reduced H3K4me3 and H4ac in fully grown oocytes. Histone 2A ubiquitination (H2Aub) and histone 3 lysine 27 trimethylation (H3K27me3) are related to gene repression, while Histone 4 acetylation (H4ac) and Histone 3 lysine 4 trimethylation (H3K4me3) are associated with the promotion of gene expression [88].

Apart from insecticides, ovarian epigenetic alternations were also found following exposure to herbicides. DNA methylation and histone modifications were found after the exposure of atrazine (ATZ) and fenoxaprop-ethyl (FE), respectively. Low-dose ATZ exposure to developing medaka (*Oryzias latipes*) induced a decrease in ovarian DNA methylation, followed by a decrease in the estrogen receptor 1 (esr1) gene. Esr1 is a gene that encodes ERα which is essential for binding to estrogen. ATZ found no transgenerational effects on ovarian medaka [91]. In contrast to other experiments, which were administrated during gestation, fenoxaprop-ethyl (FE) exposure to adult mice could also lead to histone modifications (i.e., H3K27me3 and H3K9me2) in ovaries [30].

Lastly, fungicides have also been shown to influence ovarian epigenetic changes in histone modifications, DNA methylation, and non-coding RNAs. The administration of high dose mancozeb (800 mg/kg/day) to adult mice for 4 weeks induced the methylation of histone in H3K27 [93]. In another report, changes in DNA methylation on promoters of the methylated regions (DMRs) was found in ovarian granulosa cells of F3 generation following the treatment of 100 mg/kg/day of vinclozolin to gestational rats [94,98]. In addition, differentially expressed non-coding RNAs (i.e., long non-coding RNAs (lncRNAs) and small non-coding RNAs (sncRNAs)) and mRNA, were also observed [98]. These findings refer to the transgenerational epigenetic inheritance of ovarian interferences after pesticide exposure, which is passed down throughout generations.

Epigenetic effects of pesticides on the uterus

Besides ovaries, epigenetic changes are also found in uterine rats due to pesticide exposure. Endosulfan, an insecticide, was administrated (600 µg/kg/day) to rats after birth at postnatal day (PND) 1, 3, 5, and 7, showing a decrease of DNA methylation in some ERα regulatory regions. This decrease affected the decrease of downstream genes, such as mucin 1 (MUC-1) and insulin-like growth factor 1 (IGF-1). MUC-1 and IGF-1 genes are expressed in the uterus, which involves endometrial receptivity and function, especially in embryo implantation [90]. Another pesticide that influences uterine epigenetic alterations is glyphosate (herbicide). DNA methylation and histone modifications in uterine rats altered following exposure to glyphosate (350 mg/kg/day) during gestational and lactational periods. These epigenetic modifications were a decrease of DNA methylation in the O promoter of ERα, an increase of H4ac and H3K9me3, and a decrease of H3K27me3. These results were responsible for the increase in the ERα genes [92]. The altered epigenetics in the uterus from both reports may affect infertility, implantation failure, and pregnancy complications.

Table 3: *Epigenetic effects of pesticides on the female reproductive system*

Type of pesticides	Pesticides	Organisms	Organs	Effects	Doses	Exposure	References
Insecticides	Methoxychlor (MXC)	Rats	Ovary	- Multiple loci hyper-methylation, the promoter region of ERβ, no alterations in the methylation of Erα - No change in Erα, reduced expression of ERβ	20 µg/kg/day or 100 mg/kg/day	Embryonic day (E) 19 – postnatal day (PND) 7	[97]
Insecticides	Methoxychlor (MXC)	Rats	Ovary	Epigenetic transgenerational inheritance of ovary disease (F3 generation from MXC exposed- F0 generation)	200 mg/kg	Gestational day (GD) 8 – 14	[99]
Insecticides	Endosulfan	Rats	Uterus	-Decreased DNA methylation levels in some regions of ERα regulatory -Decreased downstream gene expression, *i.e.*, MUC-1 and IGF-1	6 or 600 µg/kg/day	PND 1, 3, 5, and 7	[90]
Insecticides	Chlordecone (CD)	Mice	Ovary	-Influenced epigenetic features: decreased H4ac and H3K4me3 increased H2Aub and H3K27me3 in embryonic oocytes - Reduced H3K4me3 and H4ac in fully grown oocytes	100 µg/kg/day	E 6.5 – 15.5	[88]
Herbicides	Glyphosate	Rats	Uterus	- Changes in epigenetic features: decreased DNA methylation in the O promoter of ERα, promoter, increased H4ac and H3K9me3, and decreased H3K27me3 - ERα gene expression increased	350 mg/kg/day	GD 9 – lactational day (LD) 21	[92]

Type of pesticides	Pesticides	Organisms	Organs	Effects	Doses	Exposure	References
Herbicides	Atrazine (ATZ)	Medaka (*Oryzias latipes*)	Ovary	- DNA methylation levels decreased in the ovary of F0 females in ATZ 50 µg/L treatment, and no significant differences were observed in the ovary of F2 females - Estrogen receptor 1 (esr1) decreased in F0 but not in F2	5 or 50 µg/L	First 12 days of development with no subsequent exposure over 3 generations	[91]
Herbicides	Fenoxaprop-ethyl (FE)	Mice	Ovary	Changes in epigenetic modifications in oocytes, *i.e.*, H3K27me3 and H3K9me2	315 mg/kg/day	Adult for 30 days	[30]
Fungicides	Vinclozolin	Rats	Ovary	Differential DNA methylation at the methylated region (DMR) on the promoter in the F3 generation of granulosa cells	100 mg/kg/day	GD 8 – 14	[94]
Fungicides	Vinclozolin	Rats	Ovary	- Changes in the methylated regions (DMRs) in the F3 generation - Differentially expressed long non-coding RNAs (lncRNAs) and small non-coding RNAs (sncRNAs) in ovarian granulosa cells - Differentially expressed mRNA in ovarian granulosa cells - Transgenerational changes on the transcriptome and epigenome of ovarian granulosa cells	100 mg/kg/day	GD 8 – 14	[98]
Fungicides	Mancozeb	Mice	Ovary	Increased level of H3K27 methylation	800/mg/kg/day	Adult for 4 weeks	[93]

Abbreviations: GD: gestational day, LD: lactational day, E: embryonic day, PND: postnatal day

4. CONCLUSION

In conclusion, pesticides (e.g., insecticides, herbicides, and fungicides) have been a concern to have negative effects on the female reproductive system. The negative effects are categorized into phenotypic, genotypic, and epigenetic effects. Various types of pesticides have been proven to interfere with the female reproductive system, such as reducing the quality of the ovaries, disrupting the menstrual cycle, endometriosis, and stillbirth. The several mechanisms of action that occur are endocrine dysfunction, endocrine-disrupting chemicals, the formation of reactive oxygen species, and oxidative stress. The studies of molecular and epigenetic changes in female reproductive organs (e.g., ovary and uterus) have been obtained in various organisms, such as rats, mice, and fish. Molecular analysis revealed several key pathways that lead to the physiological changes in the reproductive system. The epigenetic modifications are found in specific regions of DNA, histone proteins, and non-coding RNAs. Furthermore, epigenetic changes not only occur in individuals but are also inherited throughout generations. These findings mean that pesticide exposure in the female reproductive system causes long-term epigenetic disruptions of the ovary and uterus, which can be associated with infertility, ovarian disease, uterine disease, implantation failure, and pregnancy complications, across generations. Future research is needed to study similar mechanisms in humans. Eventually, the use of pesticides requires strict regulation to prevent an increase in severe effects on animal and human populations.

5. CONFLICT OF INTEREST

The author declares no relevant conflicts of interest with respect to the content of this paper.

6. ACKNOWLEDGEMENTS

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REFERENCES

[1] R. W. Moore, T. A.Rudy, T. M. Lin, K. Ko, and R.E. Peterson, “Abnormalities of sexual development in male rats with in utero and lactational exposure to the antiandrogenic plasticizer Di (2-ethylhexyl) phthalate” *Environmental health perspectives*, Vol. 109, No 3, PP. 229-237, 2001.

[2] R, Margiana, S.W. Lestari, K. Alawiyah, P. Narulita, A. A. Jusuf, and, K. Ima. “Dietary Soybean (Glycine max (L.) Merr.) Improved the ZP2 Expression in Female Swiss Mice” *JBRA Assisted Reproduction*, Vol. 27, No. 2, PP. 147, 2023.

[3] D. Meirow, H. Biederman, R.A. Anderson, and, W.H.B. Wallace, “Toxicity of chemotherapy and radiation on female reproduction”. *Clinical obstetrics and gynecology*, Vol. 53, No. 4, PP. 727-739, 2010.

[4] S. Sifakis, M. Mparmpas, O.P. Soldin, A. Tsatsakis, “Pesticide exposure and health related issues in male and female reproductive system. In: Stoytcheva, M. (Ed.), Pesticides—Formulations, Effects, Fate”. *InTech, Rijeka*, Croatia, pp. 495–526, 2011.

[5] D.R. Mattison, D. R. Plowchalk, M.J. Meadows, A.Z. Al-Juburi, J. Gandy, and, A. Malek, “Reproductive toxicity: male and female reproductive systems as targets for chemical injury”. Medical

Clinics of North America, Vol. 74, No. 2, PP. 391-411, 1990.

[6] M. Ebrahimi, and M. Taherianfard, "The effects of heavy metals exposure on reproductive systems of cyprinid fish from Kor River". *Iranian journal of fisheries science*. Vol. 10, No. 1, PP.13-24, 2011.

[7] X. Wang, H. Xie, Z. Wang, and K. He, "Graphene oxide as a pesticide delivery vector for enhancing acaricidal activity against spider mites. Colloids and Surfaces" *B: Biointerfaces*, Vol. 173, PP. 632-638, 2019.

[8] C. H. King, and D. Bertsch, "Historical perspective: snail control to prevent schistosomiasis". *PLoS neglected tropical diseases*, Vol. 9, No. 4, PP. e0003657, 2015.

[9] U. Asghar, M.F. Malik, A. Javed, "Pesticide Exposure and Human Health: A Review." *J. Ecosyst. Ecography* , Vol. 01, No. 5, 2016.

[10] R.H. Hall, "A new threat to public health organochlorines and food*" Nutr. Health*, Vol. 8, PP. 33–43, 1992.

[11] R.C. Gilden, K. Huffling, B. Sattler, "Pesticides and health risks". *J. Obstet.Gynecol. Neonatal Nurs.* Vol. 39, PP. 103–110, 2010.

[12] V.I. Lushchak, T.M. Matviishyn, V.V. Husak, J.M. Storey, K.B. Storey, "Pesticide toxicity: A mechanistic approach", *EXCLI J*, Vol. 17, PP. 1101–1136, 2018.

[13] M. Ando, H. Saito, and I. Wakisaka, "Gas chromatographic and mass spectrometric analysis of polychlorinated biphenyls in human placenta and cord blood", *Env. Rer.*, Vol. 41, PP. 14–22,1986.

[14] M.S. Wolff, S. Engel, G.S. Berkowitz, S. Teitelbaum, J. Siskind, D.B. Barr, et al., "Prenatal pesticide and PCB exposures and birth outcomes". *Pediat Res*, Vol. 61, No. 2, PP. 243–250, 2007.

[15] O.J. Rando, "Daddy Issues: Paternal Effects on Phenotype", *Cell*, Vol. 151, PP. 702–708, 2012.

[16] Q. Chen, Q.C.W. Yan, and, E. Duan, "Epigenetic inheritance of acquired traits through sperm RNAs and sperm RNA modifications". *Nat. Rev. Genet*. Vol. 17, PP. 733–743, 2016.

[17] E.C. Oerke, "Crop losses to pests". *J. Agric. Sci.* Vol. 144, PP.31–43, 2006.

[18] M.M. Akashe, A.V. Nikam, and U.V. Pawade, "Classification of Pesticides : A Review". *International Journal of Research in Ayurveda and Pharmacy.* Vol. 9, No. 4, 2018.

[19] A. El Nemr, A.A. Moneer, A. El-Sikaily, A. Khaled, "PCBs and pesticides in the soft tissue of bivalves along the coast of north Egypt". *Blue Biotechnology Journal*, Vol. 1, No.2, PP, 301–313, 2012.

[20] Hassaan and Nemr, "Pesticides pollution: Classifications, human health impact, extraction and treatment techniques", *Egyptian journal of aquatic research*, Vol. 46, No. 3, PP. 207-220, 2020.

[21] Y. Abubakar, H. Tijjani, C. Egbuna, C.O. Adetunji, S. Kala, T.L. Kryeziu, J.C. Ifemeje, Iwuanyunwu, C.P. Kingsley. 2020. *Pesticides, History, and Classification*. *Natural remedies for pest, disease and weed control*, PP. 29–42, 2020.

[22] Z.J. Tano, "Identity physical and chemical properties of pesticides, in: Pesticides in the Modern World: Trends in Pesticides Analysis", *InTechopen*, pp. 1e18, 2011.

[23] I.C. Yadav, N.L. Devi, J.H. Syed, Z. Cheng, J. Li, G. Zhang, K.C. Jones, "Current status of persistent organic pesticides residues in air, water, and soil, and their possible effect on neighboring countries: a comprehensive review of India", *Sci. Total Environ*, Vol. 511, PP. 123-137, 2015.

[24] A.R. Baerwald, "Human antral folliculogenesis: what we have learned from the bovine and equine models", *Anim. Reprod*., Vol. 6, No.1,PP. 20-29, 2009.

[25] M. Puttabyatappa and V. Padmanabhan, "Developmental Programming of Ovarian Functions and Dysfunctions", *Vitam Horm.* Vol. 107, PP. 377-422, 2018.

[26] B.E. Rani, M.K. Krishnakumari, "Prenatal toxicity of heptachlor in albino rats", *Pharmacol Toxicol*, Vol. 76, PP, 112-114, 1995.

[27] J.A. Oduma, E.O. Wango, D. Oduor-Okelo, et al., "In vivo and in vitro effects of graded doses of the pesticide heptachlor on female sex steroid hormone production in rats. Comp. Biochem. Phyriol", *C. Pharmacol. Toxicol. Endocrinol*, Vol. 111, PP. 191–196, 1993.

[28] J. Boberg, H.K. Johansson, D. Franssen, M.K. Draskau, S. Christiansen, J. Cramer, and T. Svingen, Exposure to the pesticides linuron, dimethomorph and imazalil alters steroid hormone profiles and gene expression in developing rat ovaries. *Toxicology Letters*, Vol. *373*, PP. 114-122, 2023.

[29] J.K. Bhardwaj, and P. Saraf, "Malathion-induced granulosa cell apoptosis in caprine antral follicles: an ultrastructural and flow cytometric analysis". *Microscopy and microanalysis*, Vol. *20*, No.6, PP. 1861-1868, 2014.

[30] Y. T. He, L. L. Yang, Y. Zhao, W. Shen, S. Yin, and Q. Y. Sun, "Fenoxaprop-ethyl affects mouse oocyte quality and the underlying mechanisms," *Pest Manag Sci*, vol. 75, no. 3, pp. 844–851, Mar. 2019, doi: 10.1002/ps.5190.

[31] W.G. Foster, A. McMahon, E.V. Younglai, et al., "Alterations in circulating ovarian steroids in hexachlorobenzene-exposed monkeys", *Reprod. Toxicol*., Vol. 9, PP. 341–348, 1993.

[32] W.G. Foster, J.A. Pentick, A. McMahon, et al., "Ovarian toxicity of hexachlorobenzene (HCB) in the superovulated female rat". *J. Biochem. Toxicol*, Vol. 7, No. 1–4, 1992.

[33] J.A. Oduma, E.O. Wango, D. Oduor-Okelo, et al., "In vivo and in vitro effects of graded doses of the pesticide heptachlor on female sex steroid hormone production in rats", *Comp. Biochem. Phyriol. C. Pharmacol. Toxicol. Endocrinol*, Vol. 111, PP. 191–196, 1993.

[34] A. Lindenau, B. Fischer B, P. Seiler, H.M. Beier, "Effects of persistent chlorinated hydrocarbons on reproductive tissues in female rabbits". *Hum Reprod*, Vol. 9, No. 772-80, 1994.

[35] L.J. Guillete, T.S. Gross, G.R. Masson, et al., "Developmental abnormalities of the gonad and abnormal sex hormone

concentrations in juvenile alligators from contaminated and control lakes in Florida", *Environ. Health Perrp*., Vol. 102, PP. 608–613, 1994.

[36] Y. Guo, A.G. Hendrickx, and J.W. Overstreet, "Endocrine biomarkers of early fetal loss in cynomolgus macaques (Macaca farcicularir) following exposure to dioxin". *Biol. Reprod*., Vol. 60, PP. 707–713, 1999.

[37] A.P. van Birgelen, C.D. Hebert, M.L. Wenk, et al., "Toxicity of 3,3',4,4'-tetrachloroazoxybenzene in rats and mice". *Toxicol. Appl. Pharmacol.*, 156, 206–221, 1999.

[38] J.Z. Yang and W.G. Foster, "Continuous Exposure to 2,3,7,8-Tetrachlorodibenzo-P-Dioxin Inhibits the Growth of Surgically Induced Endometriosis in the Ovariectomized Mouse Treated with high Dose Estradiol*", Toxicology and Industrial Health*, Vol. 13, No. 1, PP. 15–25, 1997.

[39] R.K. Gupta, R.A. Schuh, G. Fiskum, J.A. Flaws, "Methoxychlor causes mitochondrial dysfunction and oxidative damage in themouse ovary". *Toxicol Appl Pharmacol*. Vol. 216, PP. 436–45, 2006.

[40] D. Tomic, M.S. Frech, K. Janice, J.K. Babus, R.K. Gupta, P.A. Furth, et al., "Methoxychlor induces atresia of antral follicles in ER-overexpressing mice". *Toxicol Sci*, Vol. 93, PP.196–204, 2006.

[41] H. Tatemoto, N. Sakurai, and N. Muto. "Protection of porcine oocytes against apoptotic cell death caused by oxidative stress during in vitro maturation: role of cumulus cells", *Biol Reprod* Vol. 63, PP. 805–10, 2000.

[42] A.N. Fatehi, E.C. Zeinstra, R.V. Kooij RV, B. Colenbrander, M.M. Bevers, "Effect of cumulus cell removal of in vitro matured bovine oocytes prior to in vitro fertilization on subsequent cleavage rate". *Theriogenology*, Vol. 57, No. 1347–55, 2002.

[43] A.M. Cummings, "Methoxychlor as a model for environmental estrogens", *Crit Rev Toxicol*, Vol. 27, PP. 367-379, 1997.

[44] I. Gerhard, B. Monga, J. Krähe, B. Runnebaum, "Chlorinated hydrocarbons in infertile women", *Environ Res*, Vol. 80, PP. 299-310,1999.

[45] K.L. Bruner-Tran, et al., "Dioxin may promote inflammation-related development of endometriosis", *Fertility and sterility*, Vol. 89, PP. 1287–1298, 2008.

[46] A.C. Holloway, J.J. Petrik, E.V. Younglai, "Influence of dichlorodiphenylchloroethylene on vascular endothelial growth factor and insulin-like growth factor in human and rat ovarian cells", *Reprod Toxicol*, Vol. 24, PP. 359–64, 2007.

[47] B. Simic, J. Kniewald, Z. Kniewald, "Effects of atrazine on reproductive performance in the rat", *J Appl Toxicol*, Vol. 14, PP. 401–4, 1994.

[48] T. Gojmerac, M. Uremovic, Z. Uremovic, et al., "Reproductive disturbance caused by an S-triazine herbicide in pigs", *Acta Vet Hung*, Vol. 47, PP. 129–35, 1999.

[49] L.T. Wetzel, L.G. Luempert, C.B. Breckenridge, et al., "Chronic effects of atrazine on estrus and mammary tumor formation in female Sprague-Dawley and Fischer 344 rats", *J Toxicol Environ Health.*Vol. 43, PP. 69–82, 1994.

[50] P.N. Baligar, B.B. Kaliwal, "Induction of gonadal toxicity to female rats after chronic exposure to mancozeb", *Ind Health*. Vol. 39, PP. 235–43, 2001.

[51] J.K. Bhardwaj, and P. Saraf, "Malathion-induced granulosa cell apoptosis in caprine antral follicles: an ultrastructural and flow cytometric analysis". *Microscopy and microanalysis*, Vol. *20*, No.6, PP. 1861-1868, 2014.

[52] K. Nishi, and S.S. Hundal, "Chlorpyrifos induced toxicity in reproductive organs of female Wistar rats", *Food and Chemical Toxicology*, Vol. 622013, PP. 732–738.

[53] M.H.M. Azarnia, M. Koochesfahani, Y. Rajabi, A. Tahamtani, A. Tamadon, "Histological examination of endosulfan effects on follicular development of BALB/c mice", *Bulg. J. Vet. Med*., Vol.12, No. 1, PP.33−41, 2009

[54] N.D. Koc,et al., "Dose-dependent effect of endosulfan and malathion on adult wistar albino rat ovaries". *Pakistan journal of biological sciences*, Vol. 12, No. 6, PP. 498–503, 2009.

[55] M. Guney, et al., "a. Ovarian toxicity in rats caused by methidathion and ameliorating effect of vitamins E and C". *Human & experimental toxicology*, Vol. 26, PP. 491–498, 2007.

[56] S. Medithi, Y.D. Kasa, B. Jee, K. Venkaiah, and, P.R. Jonnalagadda,"Alterations in reproductive hormone levels among farm women and their children occupationally exposed to organophosphate pesticides". *Women & Health*, Vol. 62, No. 5, PP. 454-464, 2022.

[57] P. Sood, "Imidacloprid Induced Reproductive Toxicity in Female Albino Rats". *Indian Journal of Entomology*, PP. 1-5, 2022.

[58] X. Wang, A. Anadón, Q. Wu, F. Qiao, I. Ares, Martínez-Larrañaga, M.R. Yuan, and, Z. M.A. Martínez. "Mechanism of Neonicotinoid Toxicity: Impact on Oxidative Stress and Metabolism". *Annu. Rev. Pharmacol. Toxicol*. Vol. 58, PP. 471–507, 2018.

[59] J. U. I. Iheanacho, P. I. K. Onyeka, H. I. Udujih, O. G. Udujih, & C. C. Iwuala, "Effects of sub-lethal exposure of lambda-cyhalothrin on birth outcome of female wistar rats". *World journal of pharmaceutical and medical research*. Vol. 6, No. 3, PP. 41-44, 2020.

[60] P. Wang, Y. Tian, X. J. Wang, Y. Gao, R. Shi, G. Q. Wang, and X. M. Shen, "Organophosphate pesticide exposure and perinatal outcomes in Shanghai, China" . *Environment international*, Vol. 42, PP. 100-104, 2012.

[61] Y. J. Zhou, Q. F. Qiao, L. Q. Wang, T. Y. Sheng, M. X. Cui, Q. D. Chen, and, Y. X. Zhang, "Toxicity mechanism of peri-implantation pesticide beta-cypermethrin exposure on endometrial remodeling in early pregnant mice". *Toxicology*, Vol. *489*, PP. 153497, 2023.

[62] S. E. Rier, D. C. Martin, R. E. Bowman, et al. "Endometriosis in rhesus monkeys (Macaca mulatta) following chronic exposure to 2,3,7,8- tetrachlorodibenzo-p-dioxin. Fundam. Appl". *Toxicol*, Vol. 21, PP. 433–441, 1993.

[63] L. M. Pastore, I. Hertz-Picciotto , J.J. Beaumont, "Risk of stillbirth from occupational and residential exposures".

Occup Environ Med, Vol. 54, PP. 511-518, 1997.

[64] T. E. Arbuckle, Z. Lin , L. S. Mery , "An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population". *Environ Health Perspect*, Vol. 109, PP. 851-857, 2001.

[65] J. U. I. Iheanacho, P. I. K. Onyeka, H. I. Udujih, O. G. Udujih, and C. C. Iwuala, "Effects of sub-lethal exposure of lambda-cyhalothrin on birth outcome of female wistar rats" . *World journal of pharmaceutical and medical research*.VOL. 6. No. 3, PP. 41-44, 2020.

[66] Y. Shukla, and, P. Taneja, "Mutagenic potential of cypermethrin in mouse dominant lethal assay". *Journal of environmental pathology, toxicology and oncology*, Vol. 21, No. 3, 2002.

[67] M. S. Ullah, M. Ahmad, N. Ahmad, M. Z. Khan, , and , I. Ahmad, "Toxic effects of cypermethrin in female rabbits" . *Pakistan Vet. J*, Vol. 26, No. 4, PP. 193-196, 2006.

[68] A. Abell , S. Juul , J. P. Bonde, "Time to pregnancy among female greenhouse workers". *Scand J Work Environ Health*, Vol. 26, PP. 131-136, 2000.

[69] A. J. Idrovo ,L. H. Sanin , D. Cole , J. Chavarro , H. Caceres , J. Narvaez , "Restrepo M: Time to first pregnancy among women working in agricultural production" . *Int Arch Occup Environ Health*, Vol. 78, PP.493-500, 2005.

[70] J. Varayoud, L. Monje, T. Bernhardt, M. Muñoz-de-Toro, E. H. Luque, and J. G. Ramos, "Endosulfan modulates estrogen-dependent genes like a non-uterotrophic dose of 17β-estradiol," *Reproductive Toxicology*, Vol. 26, no. 2, PP. 138–145, 2008.

[71] G. Rasier *et al.*, "Mechanisms of interaction of endocrine-disrupting chemicals with glutamate-evoked secretion of gonadotropin-releasing hormone," *Toxicological Sciences*, Vol. 102, No. 1, PP. 33–41, Mar. 2008.

[72] G. Rasier, A. S. Parent, A. Gérard, M. C. Lebrethon, and J. P. Bourguignon, "Early maturation of gonadotropin-releasing hormone secretion and sexual precocity after exposure of infant female rats to estradiol or dichlorodiphenyltrichloroethane" , *Biol Reprod*, Vol. 77, No. 4, PP. 734–742, Oct. 2007.

[73] A. L. Strong *et al.*, "Effects of the endocrine-disrupting chemical DDT on self-renewal and differentiation of human Mesenchymal stem cells", *Environ Health Perspect*, Vol. 123, No. 1, PP. 42–48, 2015.

[74] M. R. Bratton *et al.*, "The organochlorine o,p'-DDT plays a role in coactivator-mediated MAPK crosstalk in MCF-7 breast cancer cells" , *Environ Health Perspect*, Vol. 120, No. 9, PP. 1291–1296, 2012.

[75] M. R. Bratton *et al.*, "Organochlorine-mediated potentiation of the general coactivator p300 through p38 mitogen-activated protein kinase" , *Carcinogenesis*, Vol. 30, No. 1, PP. 106–113, 2009.

[76] D. E. Frigo *et al.*, "Mechanism of Ap-1-mediated gene expression by select organochlorines through the p38 MAPK pathway", *Carcinogenesis*, Vol. 25, No. 2 , PP. 249–261, Feb. 2004.

[77] C. D. Foradori, A. D. Zimmerman, L. R. Hinds, K. L. Zuloaga, C. B. Breckenridge, and R. J. Handa, "Atrazine inhibits pulsatile gonadotropin-releasing hormone (GnRH) release without altering GnRH messenger RNA or protein levels in the female rat," *Biol Reprod*, vol. 88, no. 1, Jan. 2013, doi: 10.1095/biolreprod.112.102277.

[78] G. P. Dooley, R. B. Tjalkens, and W. H. Hanneman, "The atrazine metabolite diaminochlorotriazine suppresses LH release from murine LβT2 cells by suppressing GnRH-induced intracellular calcium transients," *Toxicol Res (Camb)*, vol. 2, no. 3, pp. 180–186, 2013, doi: 10.1039/c3tx20088d.

[79] M. V. Hofmeister and E. C. Bonefeld-Jørgensen, "Effects of the pesticides prochloraz and methiocarb on human estrogen receptor α and β mRNA levels analyzed by on-line RT-PCR," *Toxicology in Vitro*, vol. 18, no. 4, pp. 427–433, Aug. 2004, doi: 10.1016/j.tiv.2003.12.008.

[80] R. Kamata, F. Sfflraishi, S. Takahashi, A. Shimizu, and H. Shiraishi, "Reproductive and developmental effects of transovarian exposure to o,p'-DDT in japanese quails," *Environ Toxicol Chem*, vol. 28, no. 4, pp. 782–790, Apr. 2009, doi: 10.1897/08-218R.1.

[81] R. Kamata, F. Shiraishi, and K. Nakamura, "Avian eggshell thinning caused by transovarian exposure to o,p′-DDT: changes in histology and calcium-binding protein production in the oviduct uterus," 2020. doi: 10.2131/jts.45.131.

[82] L. Holm, A. Blomqvist, I. Brandt, B. Brunström, Y. Ridderstråle, and C. Berg, "Embryonic exposure to o,p′-DDT causes eggshell thinning and altered shell gland carbonic anhydrase expression in the domestic hen," *Environ Toxicol Chem*, vol. 25, no. 10, pp. 2787–2793, Oct. 2006, doi: 10.1897/05-619R.1.

[83] Z. Wang *et al.*, "The fungicide Mancozeb reduces spheroid attachment onto endometrial epithelial cells through downregulation of estrogen receptor β and integrin β3 in Ishikawa cells," *Ecotoxicol Environ Saf*, vol. 208, Jan. 2021, doi: 10.1016/j.ecoenv.2020.111606.

[84] T. E. Arbuckle, Z. Lin, and L. S. Mery, "An Exploratory Analysis of the Effect of Pesticide Exposure on the Risk of Spontaneous Abortion in an Ontario Farm Population," *Environmental Health Perspectives* , vol. 109, no. 8, pp. 851–857, 2001, doi: 10.1289/ehp.01109851.

[85] S. L. Farr, G. S. Cooper, J. Cai, D. A. Savitz, and D. P. Sandler, "Pesticide use and menstrual cycle characteristics among premenopausal women in the Agricultural Health Study," *Am J Epidemiol*, vol. 160, no. 12, pp. 1194–1204, Dec. 2004, doi: 10.1093/aje/kwi006.

[86] V. F. Garry, M. Harkins, A. Lyubimov, L. Erickson, and L. Long, "Reproductive Outcomes In The Women Of The Red River Valley Of The North. I. The Spouses Of Pesticide Applicators: Pregnancy Loss, Age At Menarche, And Exposures To Pesticides," *J Toxicol Environ Health A*, vol. 65, no. 11, pp. 769–786, 2002, doi: 10.1080/00984100290071333.

[87] B. B. Bindali and B. B. Kaliwal, "Anti-implantation Effect of a Carbamate

Fungicide Mancozeb in Albino Mice," *Ind Health*, vol. 40, pp. 191–197, 2002, doi: 10.2486/indhealth.40.191.

[88] L. Legoff *et al.*, "Ovarian dysfunction following prenatal exposure to an insecticide, chlordecone, associates with altered epigenetic features," *Epigenetics Chromatin*, vol. 12, no. 1, May 2019, doi: 10.1186/s13072-019-0276-7.

[89] M. Manikkam, M. M. Haque, C. Guerrero-Bosagna, E. E. Nilsson, and M. K. Skinner, "Pesticide methoxychlor promotes the epigenetic transgenerational inheritance of adult-onset disease through the female germline," *PLoS One*, vol. 9, no. 7, Jul. 2014, doi: 10.1371/journal.pone.0102091.

[90] M. M. Milesi, J. Varayoud, J. G. Ramos, and E. H. Luque, "Uterine ERα epigenetic modifications are induced by the endocrine disruptor endosulfan in female rats with impaired fertility," *Mol Cell Endocrinol*, vol. 454, pp. 1–11, Oct. 2017, doi: 10.1016/j.mce.2017.05.028.

[91] J. A. Cleary, D. E. Tillitt, F. S. vom Saal, D. K. Nicks, R. A. Claunch, and R. K. Bhandari, "Atrazine induced transgenerational reproductive effects in medaka (Oryzias latipes)," *Environmental Pollution*, vol. 251, pp. 639–650, Aug. 2019, doi: 10.1016/j.envpol.2019.05.013.

[92] V. Lorenz, M. M. Milesi, M. G. Schimpf, E. H. Luque, and J. Varayoud, "Epigenetic disruption of estrogen receptor alpha is induced by a glyphosate-based herbicide in the preimplantation uterus of rats," *Mol Cell Endocrinol*, vol. 480, pp. 133–141, Jan. 2019, doi: 10.1016/j.mce.2018.10.022.

[93] Y. Liu *et al.*, "Protective effects of resveratrol against mancozeb induced apoptosis damage in mouse oocytes," *Oncotarget*, vol. 8, no. 4, pp. 6233–6245, 2017.

[94] E. Nilsson, G. Larsen, M. Manikkam, C. Guerrero-Bosagna, M. I. Savenkova, and M. K. Skinner, "Environmentally induced epigenetic transgenerational inheritance of ovarian disease," *PLoS One*, vol. 7, no. 5, May 2012, doi: 10.1371/journal.pone.0036129.

[95] Z. Liang, A. Riaz, S. Chachar, Y. Ding, H. Du, and X. Gu, "Epigenetic Modifications of mRNA and DNA in Plants," *Molecular Plant*, vol. 13, no. 1. Cell Press, pp. 14–30, Jan. 06, 2020. doi: 10.1016/j.molp.2019.12.007.

[96] D. J. Patel and Z. Wang, "Readout of epigenetic modifications," *Annual Review of Biochemistry*, vol. 82. pp. 81–118, Jun. 2013. doi: 10.1146/annurev-biochem-072711-165700.

[97] A. M. Zama and M. Uzumcu, "Fetal and neonatal exposure to the endocrine disruptor methoxychlor causes epigenetic alterations in adult ovarian genes", *Endocrinology*, vol. 150, no. 10, pp. 4681–4691, Oct. 2009, doi: 10.1210/en.2009-0499.

[98] E. Nilsson *et al.*, "Environmental toxicant induced epigenetic transgenerational inheritance of ovarian pathology and granulosa cell epigenome and transcriptome alterations: ancestral origins of polycystic ovarian syndrome and primary ovarian insufiency", *Epigenetics*, vol. 13, no. 8, pp. 875–895, Aug. 2018, doi: 10.1080/15592294.2018.1521223.

[99] M. Manikkam, M. M. Haque, C. Guerrero-Bosagna, E. E. Nilsson, and M. K. Skinner, “Pesticide methoxychlor promotes the epigenetic transgenerational inheritance of adult-onset disease through the female germline” , *PLoS One*, Vol. 9, No. 7, Jul. 2014.